HIGH SPEED RANDOM SEPERATION OF MICROOBJECT IN MICROCHIP BY LASER MANIPULATOR AND DIELECTROPHORESIS

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ABSTRACT

We developed a new system for high speed random separation of a microobject, such as a microbe, in the micro fluidic devise by the optical radiation pressure and dielectrophoretic force control under the microscope. An arbitrary single microbe can be isolated speedily in a microchannel, even though there are a large number of these microbes in solution. Once the target microbe is trapped at the focal point of the laser manipulator, we can easily realize exclusion of excess microbes by controlling the electric field, while keeping the target trapped at the focal point. To realize an efficient separation system, we made a new separation cell by the microfabrication. Here we show the experiment results on our system. Moreover, we propose indirect manipulation methods by the micro-tools to avoid direct laser radiation to the target. Some preliminary experiments are conducted to show the effectiveness. Our method is extremely useful in the pure cultivation of the targeted microbe and will be a promising method for biologists in discovering a new microbe or for bio-remediation.

INTRODUCTION

Recently, there has been great interest in the high throughput screening of microorganisms, for example, for finding of the novel microbes and bio-remediation. It is estimated less than about 10% within the whole in which the microorganism known at present passed. For remarkable advancement of biology and bio-engineering, it is important to develop a new technology, with which we can manipulate and separate randomly suspended microorganisms with high speed and high purity in the noninvasive manner.

For this purpose, the flow cytometer (cell sorter) is well known as a selection system for the plant cell (size: around 20 μm). However, the separation process is sequential and it takes long time to find the target object. Moreover, if the target size is reduced around 1 μm, it is quite difficult to separate it with high purity. The size of most microbe is about 2-3 μm. Thus, none of the conventional separation method is suitable for finding and discovering the unidentified microbe.

We aim to develop a selective separation system for high throughput screening of the micro- and nano-objects suspended in the liquid, such as an yeast, microbe, and DNA molecules. We integrated the laser scanning manipulator for local position control of the target, and the dielectrophoresis for exclusion of the other objects around the target by shaping the potential field in the microchannel. These different methods are cooperatively used in the microchannel chip for selective separation. Effectiveness of the basic idea was examined before[1],[2]. An arbitrary single microbe was isolated speedily in a microchannel, even though there are a large number of microbes in solution. Once the target microbe is trapped at the focal point of the laser manipulator, we can easily realize exclusion of excess microbes by controlling the electric field, while keeping the target trapped at the focal point.

To realize an efficient separation system, we made a new separation cell by the microfabrication. Configuration of the microchannel was designed to have an input port, sample injection port, extraction port, and drain port. The injection port is used to input the sample liquid, and its exit is connected to the extraction port and drain port. There is a micro-gateway at the exit of the injection port for dielectrophoretic exclusion of obstacles. By this configuration, we realized high purity separation of the target observed by the microscope. Here we show the experiment results on our system.

Moreover, we propose indirect manipulation methods by the micro-tools. There have been reported that direct irradiation of the laser beam may give some damage to the trapped object. This phenomenon depends on the target, wave length, and power of the laser. To evade this problem, we proposed the indirect manipulation of the target by the laser trapped micro-tools. Some preliminary experiments are conducted to show the effectiveness.

SELECTIVE SEPARATION METHOD

For the selective manipulation of the microbe, whose size is less than around 10 μm, the non-contact type manipulation method is suitable, since we can reduce the disturbance. There are several different approaches in the style of force exertion on the object.

We can classify the non-contact manipulation as follows on the point of force action.

(1) Point force
    Laser manipulator using optical trap [1]-[5]
(2) Multiple points force
    Ultrasonic [6]
(3) Line force
    Capillary flow
    Coulomb force
(4) Field (space) force
    Dielectrophoretic force [7]-[10]

Each method has particular merit.

For separation of the cell, the flow cytometer (cell sorter) is widely used. The principle is based on the combination of liquid drop flow and Coulomb force. Sorting speed of the cell is really fast(~ 5000/s), however, the separation process is sequential and the object is normally bigger than the microbe.

Here we aim to develop a new system, which can extract a microbe selected under the microscope. None of the conventional methods are appropriate for the selective, high speed and efficient separation of the microbe. So, we proposed to integrate the following different methods to meet our demands [1].

(i) The laser scanning manipulator utilizing the optical trapping for local control [5]

It can trap the microbe at the focal point and manipulate the target by controlling the position of the focal point.

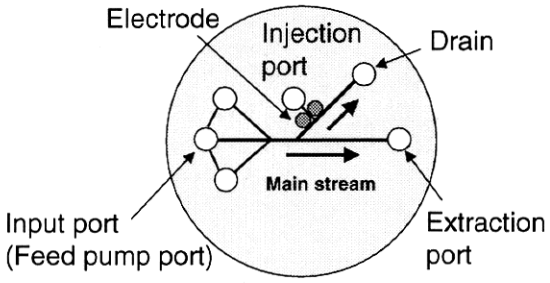
(ii) The dielectrophoretic manipulation utilizing the electric field gradient force for exclusion of the other objects around the target.

We have studied the transportation system of DNA molecules for the purpose of transporting and separating a single DNA molecule by use of the dielectrophoretic force in the Micro DNA Flow System[10]. Based on this method, we can easily realize non-contact exclusion of unneeded microbes by controlling the electric field around the target. Here we propose a micro-gateway inside the microchannel.

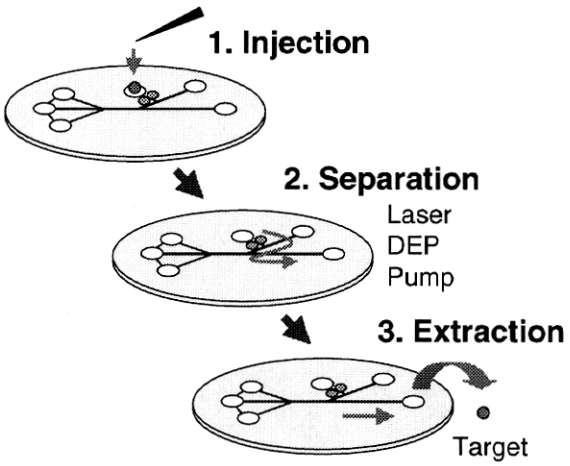
(iii) Capillary flow in the microchannel made by the pump to extract the isolated target without fault.

Capillary flow is suitable for long distance transportation of the target. Here we propose a new microchannel design.

There are a number of microbes suspended in aqueous solution. Normally, the selective manipulation is quite difficult in such case. Figure 1 shows the conceptual figure of the proposed selective separation method of the microbe in the microchannel on the chip. Configuration of the microchannel was designed to have an input port (feed pump port), injection port, extraction port, and drain port. There is uniform stream (main stream) from the input port to the extraction port and the drain port. The injection port is used to input the sample liquid, and its exit is connected to the extraction port and drain port. There is a micro-gateway at the exit of the injection port for dielectrophoretic exclusion of obstacles. The target is observed by the microscope and is manipulated by the laser manipulator to the main stream to get out from the extraction port. The obstacles are hindered to go to the extraction port by the micro-gateway and counter flow to the drain port. Some are forced to go to the drain port. By this configuration, we can realize high purity separation of the target observed by the microscope. Here the optical trapping force is big enough to manipulate the target under this environment. The proposed methodology is suitable for manipulation of an arbitrary biological particle. The laser scanning manipulator and the dielectrophoretic manipulation are integrated, and the target is isolated and selectively guided to the capillary stream for extraction.



(a) Example of the separation chip (Top view)



(b) Separation process

Figure 1: Concept of the selective separation in the microchannel on the chip

SELECTIVE SEPARATION SYSTEM

We developed a new system of random extraction of a microbe by integrating the optical trapping and dielectrophoretic force inside the micro fluidic device. Figure 2 shows the schematic diagram of the laser scanning manipulator. It has two laser beams.

Focus of the A Laser (wave length: 832 nm, power 200 mW) is scanned by the Galvano mirrors. The laser power at the focus through the system is 8 mW. The laser trapping force of the A laser is measured in Fig. 3. In this case, a polystyrene bead (diameter: 3 μm) was trapped by the A laser and the trapping force was evaluated by the storks force as follows[11].

$$F_s = \frac{6\pi\eta a U}{\left\{1-\frac{9}{16}\left(\frac{a}{h}\right)+\frac{1}{8}\left(\frac{a}{h}\right)^3-\frac{45}{256}\left(\frac{a}{h}\right)^4-\frac{1}{16}\left(\frac{a}{h}\right)^5\right\}} \quad (1)$$

where

η: Fluid viscosity
a: Radius of the particle
U: Fluid velocity
h: Distance from the surface

The maximum velocity of the bead is 140 μm/s at 6μm height from the bottom. The trapping force of the A laser is estimated 8.8 pN.

Focus of the B laser (wave length: 690 nm, power 30 mW) is fixed, but is scanned by the XYZ stage. The laser power at the focus through the system is 3 mW.

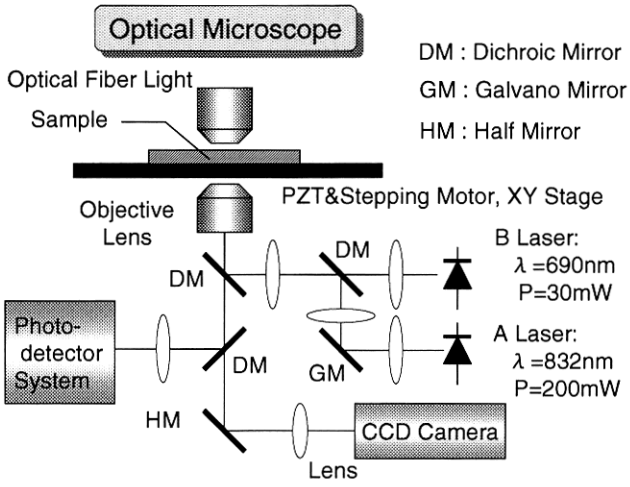


Figure 2: Schematic diagram of the laser scanning manipulator system

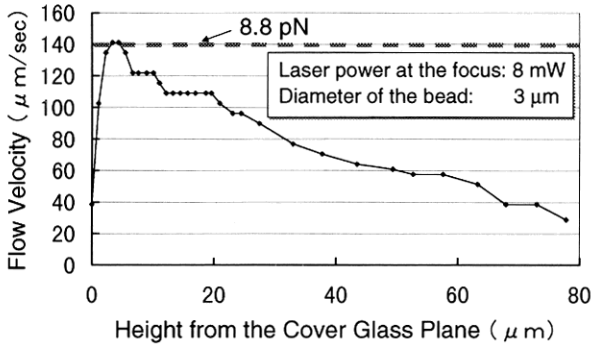
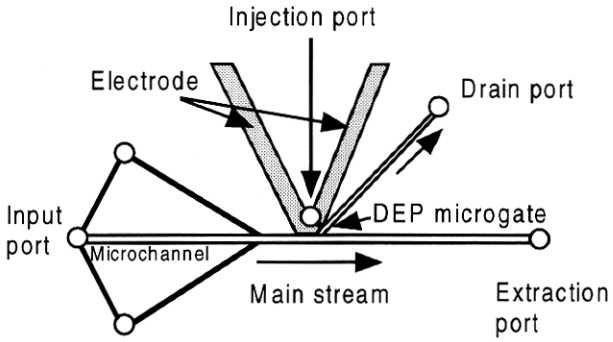
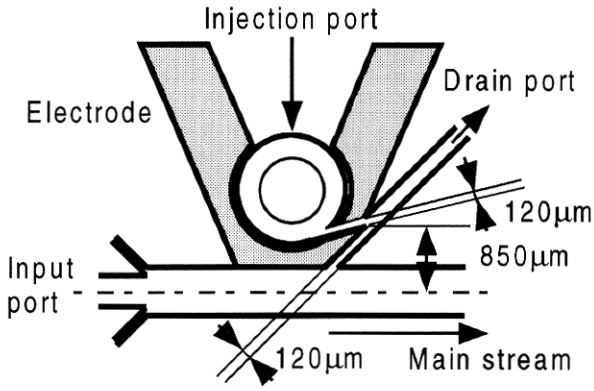


Figure 3: Measurement of the A laser trapping force



(a) Microchannel and ports allocation



(b) DEP micro-gateway

Figure 4: Design example of the separation cell

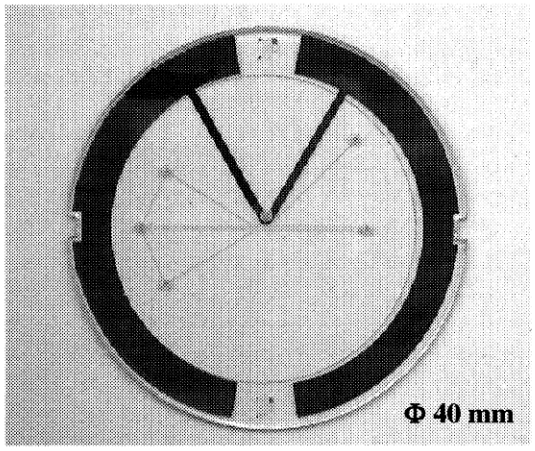
We aim to take out the target microbe in 1 minute. So, we designed a new microchannel. Figure 4 shows an example. High frequency voltage is applied between the electrodes. Once the sample liquid is injected, passage of the obstacles is hindered by the dielectrophoresis (DEP) around the gap between the electrodes. There is an uniform stream from the input port to the extraction port and the drain port. The obstacles escaped from the DEP micro-gateway are forced to go to the drain. The laser manipulator transports the target to the main stream to send it out.

The microchannel is made by the chemical etching process(HF) of glass. Figure 5 shows one example of separation chip design. It has electrodes shown in Fig. 5 (b). The distance between the DEP micro-gateway and the main stream is 850 μm. The separation cell is installed in the attachment, which is shown in Fig. 6, and is connected with tubes.

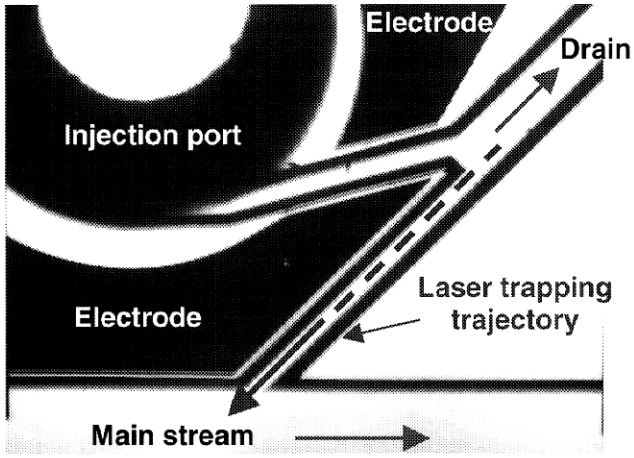
SEPARATION EXPERIMENT

In the experiment, we applied 46Vp-p, 1MHz between the electrodes. The yeast cells(size: 4-5 μm), for example, are drawn to the electrodes as in Fig. 7. The optical trapping power is strong enough to guide the target object to the main stream. The laser trapped target is moved against the flow in 30μm/s by the B laser, and

it can be extracted within 1minute successfully. We can reduce extraction time by increasing the laser manipulation speed and modifying the configuration of the microchannel around the micro-gateway and the entrance to the main stream.



(a) Separation cell made by microfablication



(b) Close-up photograph of the electrodes in the cell

Figure 5: Photograph of the separation cell

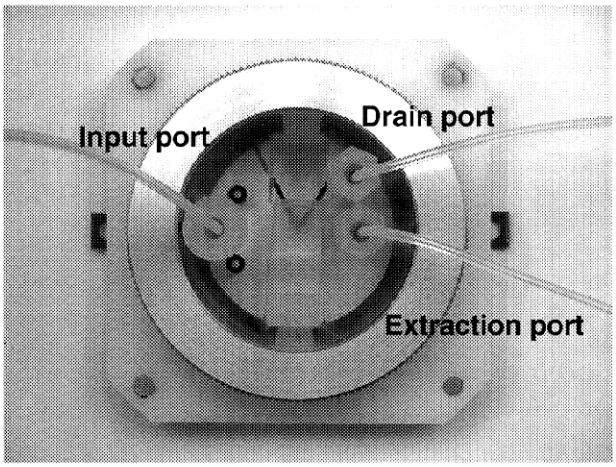


Figure 6: Attachment of the separation cell with connected tubes

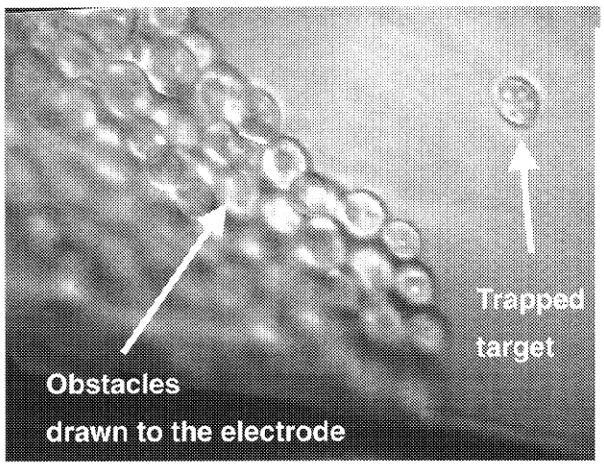
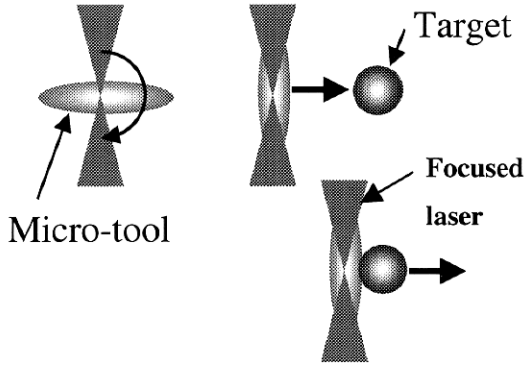
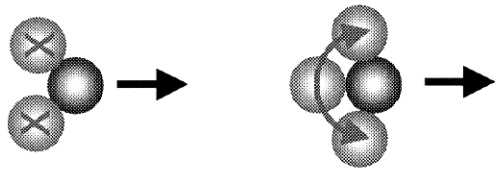


Figure 7: Obstacles drawn near the electrode by the dielectrophoresis and the target Yeast cell trapped by the laser in the microchannel

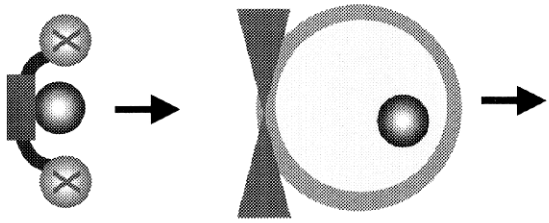


(a) Micro-tool: side view (One point contact)



(b) Micro-tools: top view (Multiple points contact by multiple beams)

(c) Micro-tools: top view (Multiple points contact by scanned beams)



(d) Micro-basket: top view (plane contact)

(e) Micro-capsule: side view (encapsulation)

Figure 8: Classification of the indirect laser manipulation

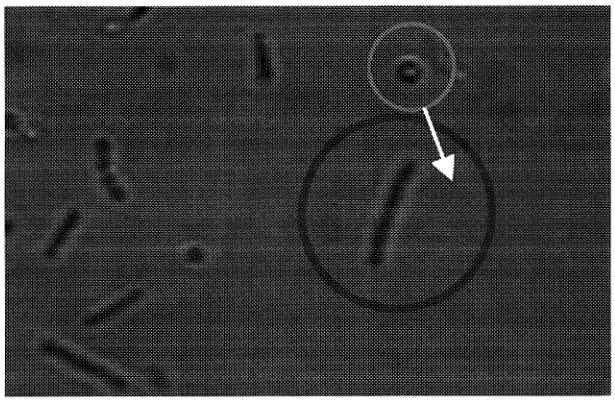
INDIRECT MANIPULATION

Next we tested the indirect manipulation of the microobject. There have been reported that direct irradiation of the laser beam may give some damage to the trapped object[12]. This phenomenon depends on the target, wave length, and power of the laser. To evade this problem, we proposed the indirect manipulation of the target by the laser trapped micro-tools. The method is classified as follows and shown in Fig.8.

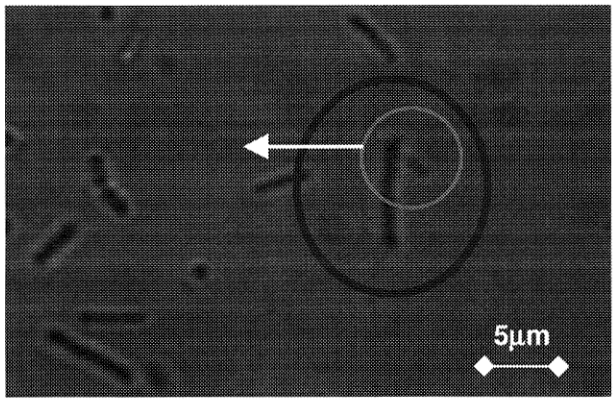
(1) Pushing by the micro-tool(s)
  one point contact
  multiple points contact
  plane contact (ex: by the micro-basket)
  etc.
(2) Encapsulation in the micro-capsule
  (ex: in the liposome)

As a micro-tool, we tested a Lactobacillus bulgaricus (LB) and a polystyrene bead as a micro-chopsticks and a liposome as a micro capsule.

Figure 9 shows an example of the indirect laser manipulation by a single micro-tool. In this case, we used LB as a micro-tool. In this figure, the target LB was pushed by the laser trapped LB. There is no remarkable interference between the target and the tool.



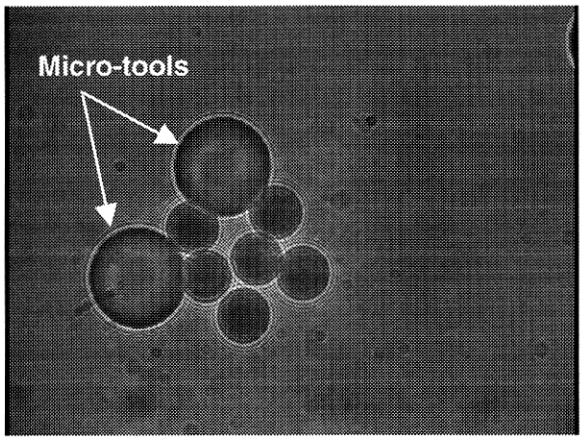
(a) LB tool trapped by the laser



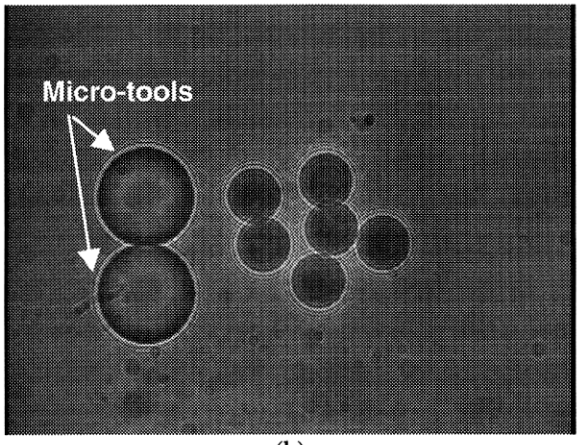
(b) LB target pushed by the trapped LB

Figure 9: Indirect manipulation of LB by the laser trapped LB (LB tool)

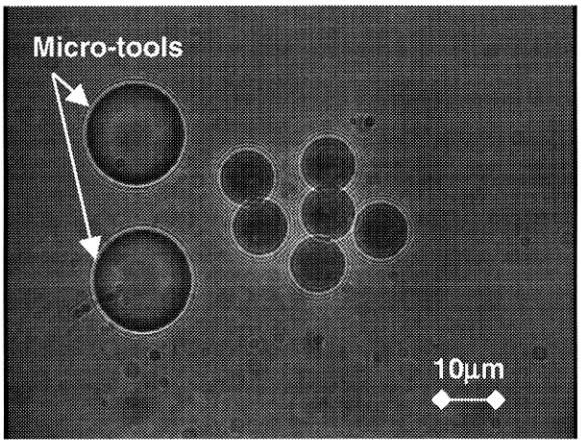
Figure 10 shows an example of the indirect laser manipulation by two micro-tools. In this case, we used two polystyrene beads (diameter: 20 μm). One bead was trapped by the A laser and the other by the B laser. Each XY positions are controlled independently. Thus, these two beads can be used as a chopsticks. In this figure, small beads (diameter 10 μm) were collected in the middle by the micro-tools.



(a)



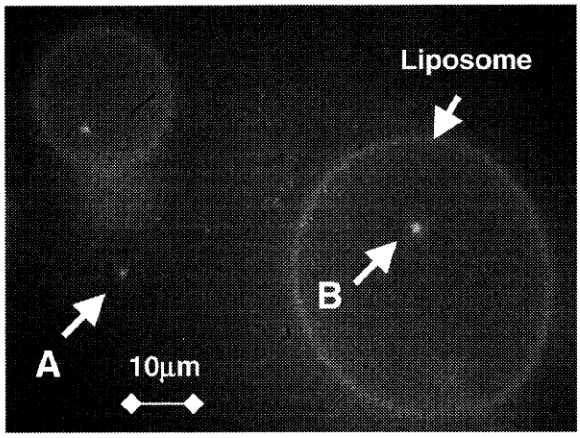
(b)



(c)

Figure 10: Indirect laser manipulation by two micro tools

Figure 11 shows an example of the indirect laser manipulation by the micro-capsule. In this case, we used liposome as a micro-capsule. The T4 DNA molecule was encapsulated in the liposome and the capsule was manipulated successfully.



A: Globule state DNA outside the liposome
B: Globule state DNA inside the liposome
Figure 11: T4 DNA molecule inside the liposome (Cooperation with Prof. Yoshikawa, Kyoto Univ.)

DISCUSSION AND CONCLUSIONS

A new system for random separation of microobject was proposed. An arbitrary selected single microbe was extracted speedily with high purity, even though there are a large number of microbes in solution. We succeeded in extracting the Yeast cell within 1 minutes. There is direct laser irradiation to the target while the laser manipulator transports the target from the micro-gateway to the entrance of the main stream. We cultivated the extracted Yeast cell and it was proliferated. For the purpose of Yeast cell extraction, the system is effective. However, we are not sure, in general, whether the direct laser irradiation to the target is safe or not. The safety depends on the target, wave length, and power of the laser. We need to investigate on this issue in our future.

On the other hand, we proposed the indirect manipulation of the target by the laser trapped micro-tools. We classified the indirect manipulation methods. We will be able to extend our idea with the new microfabrication methods. In this paper, we demonstrated some cases and showed effectiveness. The micro-tools can be prepared outside the separation cell and injected with the sample liquid, or assembled inside the cell.

Exploration of the microbes will be encouraged in the 21st century for effective utilization of earth resource. We confirmed the effectiveness of the proposed method. Our method will be extremely useful in the pure cultivation of the targeted microbe and will be a promising method for biologists in discovering a new microbe.

ACKNOWLEDGEMENTS

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